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GABA-induced response in spiral ganglion cells acutely isolated from guinea pig cochlea

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Abstract

The physiological and pharmacological properties of γ-aminobutyric acid (GABA)-induced responses were investigated in acutely isolated spiral ganglion cells (SGCs) of guinea pig by using either a nystatin-perforated patch recording configuration or a conventional whole-cell patch recording mode combined with rapid drug application. GABA and $GABA_A$ subtype receptor agonist, muscimol, induced inward currents in a concentration-dependent manner in 74% of all cells. The current-voltage relationship for the GABA response indicated the GABA-induced current in SGCs is carried by Cl^-. Bicuculline (BIC), strychnine (STR), and picrotoxin (PTX) suppressed the GABA response in a concentration-dependent manner. BIC and STR, and PTX blocked the GABA response in a competitive manner and in a non-competitive manner, respectively. For inorganic antagonists, Cd^{2+} and Ni^{2+} also inhibited the GABA response. On the other hand, Zn^{2+} failed to suppress the GABA response in SGCs. An antibiotic, benzylpenicillin, suppressed the GABA response. The GABA response was augmented by both a barbiturate derivative, pentobarbital (PB), and a benzodiazepine derivative, diazepam. The results suggest clearly that the physiological and pharmacological characteristics of $GABA_A$ receptor on acutely isolated guinea pig SGCs are quite similar to the common $GABA_A$ receptor found in other sensory ganglion cells.

Keywords: GABA; Spiral ganglion; Transmitter; Patch-clamp; Guinea pig

1. Introduction

Sounds are transduced by hair cells into electrical activity. The signals are transmitted to primary afferent neurons, which conduct them to the cochlear nucleus in the central nervous system. Investigations in physiological properties of afferent auditory fibers have ever been mainly performed on the spike activities (Geisler, 1998). The spiral ganglion cells (SGCs), those are the soma of afferent auditory neurons, were used in order to study the auditory neurons at the single cell level. Santos-Sacchi (1993) and our laboratory (Nakagawa et al., 1991a; Hisashi et al., 1995; Shimozono et al., 1995) used the acutely isolated SGCs to investigate the voltage-dependent channels and chemically-gated channels by the patch-clamp technique or Ca^{2+}-sensitive dye. The membrane channel properties and the mathematical modeling were reported in the cultured SGCs isolated from embryo and neonatal mice or rat cochlea (Malgrange et al., 1997; Lin and Chen, 2000; Lin et al., 2000; Lin and Hant, 2001).

There are two major efferent systems in the mammalian cochlea (Geisler, 1998). One is the medial efferent system of which neurons originate from a medial portion of the superior olivary complex (MOC) and contralaterally innervate upon the outer hair cells (OHCs) (Geisler, 1998). Another is the lateral efferent system. Neurons originate from a lateral portion of the superior olivary complex (LOC) and ipsilaterally innervate on the afferent neurons under the inner hair cells (IHCs). The immunohistochemical studies suggested several candidate neurotransmitters in the lateral system (Eybalin,

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1993). γ-Aminobutyric acid (GABA) is one of the candidates. Glutamic acid decarboxylase (GAD)-like immunoreactivity, a marker of GABAergic neuron, was reported in some of neurons in the efferent systems of guinea pig and rat using a light microscopic study (Fex and Altschuler, 1984). At the ultrastructural level, GABA-like immunoreactivity was clearly restricted to some of the axons and the terminals filled with numerous labeled vesicles in the efferent systems (Fex and Altschuler, 1984; Usami et al., 1988). The microiontophoretic application of GABA into the synaptic region of the IHCs reduced the glutamate (Glu)-induced firing rate of afferent fibers recorded extracellularly (Felix and Ehrenberger, 1992). Both $GABA_A$ and $GABA_B$ responses were recorded in cultured SGCs dissected from the cochlea under development (Malgrange et al., 1997; Lin et al., 2000).

We aimed to record the GABA response from SGCs acutely isolated from matured mammalian cochlea. The direct action of GABA on the SGCs was investigated by the use of the patch-clamp technique in either the nystatin-perforated patch configuration (Horn and Marty, 1988) or the conventional whole-cell patch recording mode (Hamill et al., 1981) combined with a rapid drug application method (Murase et al., 1989). Preliminary version of this work has been reported elsewhere (Yamashita et al., 1995; Nakagawa et al., 1996).

2. Materials and methods

2.1. Isolation of spiral ganglion cells

Isolation procedure was similar as previously reported (Nakagawa et al., 1991a). Guinea pigs (albino, either sex, 200–800 g) with intact Preyer reflexes were used. The temporal bones were removed after quick decapitation under ether anesthesia. The modiolus was removed and treated enzymatically in a Krebs solution containing 500 IU/ml dispase (Godo Shusei, Japan) at 31 °C for 5–10 min. Thereafter, the modiolus was cut perpendicularly to the axis and the turns separated, usually yielding four pieces. The osseous laminae were destroyed mechanically and the cells were dissociated by gentle pipetting with fine fire-polished pipette of which the tip diameter was between 120 and 170 μm under binocular microscope. The SGCs of each turn were dissociated in separate chambers. The SGCs of guinea pig are classified into two types, types I and II cells (Brown, 1987). Some cells could be distinguished as type I because of remaining myelin sheaths. However, in the cells without myelin sheaths it was difficult to determine whether they were type I or type II cells. The myelin sheaths were possibly removed by the mechanical procedure. Since the cells in which the soma membrane was partially exposed were preferentially used in the present experiments, these cells are type I cells because of their remnants of a myelin sheath. The data were obtained from 125 dissociated SGCs.

2.2. Experimental solutions

The compositions of solutions were as follows. The standard external solution contained (in mM) 150 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), and 10 glucose. In the reversal potential experiments, KCl was replaced by CsCl. Nominally Ca^{2+}-free external solution contained 150 NaCl, 5 KCl, 5 $MgCl_2$, 10 HEPES, 2 ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), and 10 glucose. The pH for external solutions was adjusted to 7.4 with Tris–OH. A nystatin stock solution consisting of 10 mg/ml nystatin in methanol was prepared. Before beginning the experiments, the stock was diluted with the pipette solution to give a final nystatin concentration of 100–200 μg/ml. The internal solution for a perforated-patch configuration consisted of 150 KCl and 10 HEPES. For the reversal potential experiments, KCl in both external and internal solution was replaced by equimolar CsCl. In using various internal Cl^- concentrations, for 80 mM and 30 mM Cl^- internal solutions 70 mM and 120 mM CsCl were replaced by equimolar Cs-gluconate, respectively. For the whole-cell patch recording mode, internal solution contained (in mM) 150 KCl, 0.5 $CaCl_2$, 5 EGTA, 2 ATP-Mg, 0.2 cAMP, 0.2 GTP-Na and 10 HEPES. The pH for all internal solutions was adjusted to 7.3 with Tris–OH.

2.3. Electrical measurements

The method for the whole-cell patch recording has been described elsewhere (Hamill et al., 1981; Nakagawa et al., 1991a). The nystatin-perforated patch configuration was used in most of the present experiments (Horn and Marty, 1988). Recordings were made using a List Medical EPC-7 patch-clamp amplifier. Currents were filtered by a low pass filter with a cut-off frequency of 1 kHz. Data were recorded on a pen recorder and stored by video recorder after A/D conversion for further analysis. After forming a GΩ seal, the pipette potential was set to −70 mV, and a 10 mV voltage pulse was periodically delivered to monitor the access resistance. The experiments were performed after the access resistance reached a steady level of 10–20 MΩ. The value for compensation of the series resistance was between 50 and 75% by the use of the internal compensation circuit. All experiments were performed at room temperature (20–23 °C).

2.4. Drugs

The drugs employed in the present experiments were nystatin, adenosine 5′-triphosphate (ATP) (magnesium salt), adenosine 3′,5′-cyclic monophosphate (cAMP), guanosine 5′-triphosphate (GTP) (sodium salt), GABA, muscimol, bicuculline (BIC), picrotoxin (PTX), and strychnine (STR; Sigma), and pentobarbital (PB) sodium salt (Ishizu), and L-glutamate (Tokyo Kasei). Benzylpenicillin (PCG) and diazepam (DZP) were presented by Meiji and Yoshitomi Pharmaceutical Co., respectively. All drugs were dissolved in solutions just before use, and the osmolarity of external solutions was kept constant by adding sucrose. Drugs were applied by using a rapid Y-tube application method (Murase et al., 1989).

3. Results

3.1. GABA response in SGCs

Fig. 1A shows that the dissociated spiral ganglion cells of guinea pig responded to GABA at a holding potential (V_H) of −70 mV under the voltage-clamp conditions. Efforts were made to first record the GABA response in the ruptured whole-cell recording mode. To check recovery from desensitization of the GABA receptor, 3×10^{-5} M GABA was applied every 5 min. Although nearly the same amplitude of 10^{-4} M Glu response could be recorded, a remarkable run-down of the GABA-induced current was observed even without a breakdown of the holding current. The relative values of the GABA response and the Glu response 30 min after the first application of each drug were 13 ± 12% and 96 ± 18%, respectively. Cells without holding current breakdown for at least 30 min were employed to calculate these ratios ($n = 8$). Next, a nystatin perforated-patch configuration was used. In this configuration, run-down of the current amplitude was much less in 30 min (102 ± 15%, $n = 9$) than that in the conventional whole-cell patch recording mode. Since stable responses to GABA were obtained in the nystatin perforated-patch configuration, most of the present experiments were performed in this mode except where otherwise noted.

The GABA response could not be recorded from all SGCs with successfully achieved perforated-patch configurations.